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10/797,019

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EXAMINER

GOUGH, TIFFANY MAUREEN

ART UNIT

PAPER NUMBER

1657

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PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 10/797,019	Applicant(s) SAVILLE ET AL.	
	Examiner TIFFANY M. GOUGH	Art Unit 1657	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 17 March 2008.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 2-7, 11-14, 31-36 and 39-65 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 2-7, 11-14, 31-36 and 39-65 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413) |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | Paper No(s)/Mail Date. _____ |
| 3) <input checked="" type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08) | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| Paper No(s)/Mail Date <u>3/17/2008</u> . | 6) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

Applicant's response, IDS and Declaration filed 3/17/2008 has been received and entered into the case. Claims 2-7,11-14, 31-36,39-65 are pending and have been considered on the merits. All arguments and amendments have been considered.

Claim Objections

Claims 2-7,11-14 are objected to because of the following informalities: These claims appear to be dependent upon claim 31, however, applicants amendment introduces confusion wherein it reads " A method as defined in claim 3 1..." Therefore, as written the claims truly depend on claim 3. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 34,35,41,45,61,62,65 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter, which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Specifically, claims 34,61 require that "the diluted enzyme exhibit at least the same level of enzyme activity per equal volume of the ...", this introduces new matter. Claims 35,45,62 state that the solution is enhanced by at least 200%, this also introduces new matter. Claims 41,65

require that the pH of the solution maintains enzyme activity, this additionally introduces new matter.

Applicant states on p.1 of the argument submitted 3/17/2008 that the basis for claims 34 and 35 can be found by combining the teachings on page 4, lines 15-16 of the specification, however, there is no such support for the claims on p.4. Applicant also states support for claims 41 can be found on page 1, line 26 of the specification, however, no such support can be found. **This is a new matter rejection.**

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 2-7,11-14,31,34,42,46-65 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

It is unclear what is meant by "raw enzyme and raw enzyme weight." Although, applicant defines "raw enzyme and raw enzyme weight" it is suggested that "crude enzyme" may better describe applicant's invention. Applicant states that "raw enzyme solution" is defined by their specification, while this is true, applicant refers to the raw enzyme solution as the crude enzyme solution in the specification. Further, applicant fails to present "enzyme weight" in a standard form of activity as is known in the art and as recommended by Methods of Enzymology. A clarification with respect to applicant's enzyme method is advised.

In addition, claims 34 and 61 do not distinctly claim applicants invention. Applicant claims that the diluted solution exhibits the same level of enzyme activity...of the enzyme solution before dilution. However, applicant does not distinctly claim which diluted solution has this enzyme activity, i.e. they do not direct one to any specific step in the method of claim 31, thus the diluted solution according to claims 31 and 64 have not been treated with activated carbon. Therefore, there is no scientific evidence to support that a diluted solution alone would have the same activity as a solution before dilution.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 1-6,9,10, 31-33,39,40,42-43,44,46-52,63-65 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lausten et al (US2002/0020668 A1).

Applicant claims a method of enhancing the intrinsic activity of an enzyme solution, preferably a hydrolase such as amylase, glucoamylase and cellulase, by treating with a purifying agent, activated carbon. The raw enzyme solution is diluted with water and removed by filtration. Such method may also be carried out through a column. The enzyme to carbon ratio is not to exceed 50:1, preferably 15:1. Additionally that if any cells are present in the solution, that they are filtered out.

Lausten teach the use of activated carbon in a fermentation broth, particularly with enzymes such as amylases and cellulases, to remove soluble impurities by purification improving the quality of a product (see abstract and 0015,0018-0046). The carbon is added at concentration of up to 2% w/w (see 0046). The enzyme solutions are further diluted with water before addition of carbon and further microfiltered (see examples 1 and 2), although they also teach the purification of such enzyme solutions with activated carbon may also be performed by such methods such as ultrafiltration, chromatographic methods, i.e. column method, adsorption and/or crystallization (see 0057). Lausten also teaches that the fermentation broth may be treated prior to the method by separating out solids by filtration, flocculation or centrifugation, i.e. removing cells if present (0043 and 0044).

Although the above references do not specifically state the enhancement of the enzyme activity, the method of treating a diluted enzyme solution with a purifying agent, activated carbon, is the same. Lausten does not teach the exact dilution amounts and ratios. However, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical. “[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.” In re Aller, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955) (Claimed process which was performed at a temperature between 40°C and 80°C and an acid concentration between 25% and 70% was held to be prima facie obvious over a reference process which differed from the claims only in

that the reference process was performed at a temperature of 100°C and an acid concentration of 10%.); see also Peterson, 315 F.3d at 1330, 65 USPQ2d at 1382 (“The normal desire of scientists or artisans to improve upon what is already generally known provides the motivation to determine where in a disclosed set of percentage ranges is the optimum combination of percentages.”). See MPEP 2144.05.

Claims 1-6, 12,13, 31-33,39,40,42,43,44,46-61,63-65 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lausten et al (US2002/0020668 A1) in view of Shenoy et al (J. of Bioscience, vol 7, 1985) and http://www.ap-lab.com/circular_dichroism.htm.

Applicant claims a method of enhancing the intrinsic activity of an enzyme solution by treating with a purifying agent. The enzyme solution of enhanced activity is claimed to have a relative absorbance intensity lower than the raw enzyme solution, preferably in the CD spectral range of 205-230 nm. Applicant further claims the enzyme to be alpha-amylase.

As stated above, Lausten teach the use of activated carbon in a fermentation broth, particularly with enzymes such as amylases and cellulases, to remove soluble impurities by purification improving the quality of a product (see abstract and 0015,0018-0046). The carbon is added at concentration of up to 2% w/w (see 0046). The enzyme solutions are further diluted with water before addition of carbon and further microfiltered (see examples 1 and 2), although they also teach the purification of such enzyme solutions with activated carbon may also be performed by such methods such as ultrafiltration, chromatographic methods, i.e. column method, adsorption and/or

crystallization (see 0057). Laustsen also teaches that the fermentation broth may be treated prior to the method by separating out solids by filtration, flocculation or centrifugation, i.e. removing cells if present (0043 and 0044).

Laustsen does not teach a CD spectral ranges. Shenoy et al (J. of Bioscience, vol7,1985) teach the purification of glucoamylases. They teach that the catalytic activity of a protein, i.e. enzyme is related to its "active" conformation, i.e. secondary and tertiary structure. The specific activity of the purified enzymes was three times higher than that of the original non-purified glucoamylase (see p.400). They teach that the UV (CD) spectra of glucoamylases from 3 species show peaks at 289-293,279-282 and 257-259 nm (see p.400-402), but also reveal negative bands at 217-220,208-210 (see p. 402).

Shenoy does not teach lower CD spectrum ranges such as those claimed by applicant nor alpha-amylase. Information found at http://www.ap-lab.com/circular_dichroism.htm teaches that any change in structure of proteins will affect the CD spectral range, therefore a change in the spectral range appears to be an inherent property of purification, i.e, structural change, of a protein. Thus, one of ordinary skill in the art would be motivated and it would therefore be obvious to claim a CD spectral range lower than that of a raw enzyme solution given that a change in structure ultimately affects the CD spectrum. When purifying a protein such as enzymes, one would have a reasonable expectation of success in obtaining a CD spectrum range lower than that of the raw enzyme solution given that purification, enhancing the catalytic activity of an enzyme, ultimately alters the secondary and

tertiary structure, therefore altering the CD spectrum range. Further, it would be obvious to optimize these parameters through routine experimentation.

Also it would be obvious to use other hydrolase enzyme such as alpha-amylase because Lausten teach the use of activated carbon in a fermentation broth, particularly with enzymes such as amylases and cellulases, to remove soluble impurities by purification improving the quality of a product (see abstract and 0015,0018-0046). Therefore, one of ordinary skill in the art at the time of the invention would have been motivated to purify an enzyme such as alpha-amylase with activated carbon as taught by Lausten and would have a reasonable expectation of success in obtaining a CD spectral range lower than that of the raw enzyme solution given what is known in the art of the change in structure by purification of a protein.

Claims 1-6, 11,14, 31-33,39,40,42,43,44,46-58,64,65 are rejected under 35 U.S.C. 103(a) as being unpatentable over Aikat et al (Biotechnology Letters, vol 23, 2001,p.295-301) in view of Lausten et al (US2002/0020668 A1).

Applicant claims a method of enhancing the intrinsic activity of an enzyme solution, specifically a hydrolase, by treating with a purifying agent, activated carbon, and further removing the activated carbon from the enzyme solution by centrifugation. The purified enzyme solution is said to have a CD and UV distinct from that of the raw enzyme solution, specifically 30 nm less and the enzyme to carbon ratio is not to exceed 15:1.

Aikat et al teach the purification of protease by activated charcoal, i.e. activated carbon. They demonstrate the purification by activated charcoal in terms of fold purification and by electrophoretic analysis (see introduction). The enzyme solution was mixed with activated charcoal and allowed to react for a specific period of time prior to centrifugation, thus removing the activated carbon, at which time the supernatant was examined by spectroscopy. Further analysis was carried out by electrophoresis (see p. 296). The enzyme solution (1 ml) was treated with 50 to 150 mg of activated charcoal, although 75 mg of charcoal was selected as their optimum ratio. By gel analysis they observed the removal of almost all of the smaller proteins, confirming the purifying action of activated charcoal.

Further, Aikat diluted the crude enzyme solution 10 times to bring its absorbance within the range of that of charcoal-treated enzyme, which shows distinct troughs at 260 nm and a peak at 280nm. In the crude diluted solution there appeared to be a peak at 260 nm and no valley (see p.299 to 300).

Aikat does not teach diluting the raw enzyme solution prior to treating with a purifying agent. However, as stated above Lausten teach diluting an enzyme solution prior to treating the activated carbon. Thus, at the time of the invention it would have been obvious to one of ordinary skill in the art to dilute an enzyme solution prior to treatment because the prior art teaches dilution prior to treatment with activated carbon. Moreover, at the time of the claimed invention, one of ordinary skill in the art would have been motivated to have diluted an enzyme solution prior to purification with activated

carbon with a reasonable expectation for successfully enhancing the intrinsic activity of such solution because the art teaches such success when using the claimed enzyme solution and purifying agent.

Response to Arguments

Applicant's arguments filed 3/17/2008 have been fully considered but they are not persuasive. Applicant argues that the Declaration submitted demonstrates that the claimed invention results in a surprising 200-900% increase in enzyme activity. Applicants declaration does not appear to be commensurate in scope with the claimed invention or what is disclosed in applicants specification. Applicant is comparing the enzymes, Liquozyme and Allzyme, and their effects on ethanol production and glucose conversion. Applicants third comparison is similar to the second, however, comparing the effects of diluting glucoamylase and its effects on maltodextrin conversion. These comparisons are not commensurate in scope with the claimed invention and introduce confusion as to what "activity" is actually being increased. Is the actual measured activity measured in U/ml or is it the enzymes ability to convert starches and how are those activities intrinsic?

Secondly, applicant argues that Lausten's broth cannot be processed through activated carbon by the claimed method due to the presence of cells. Lausten clearly teaches a filtration step to remove cells. Further, it is well known in the art that cells and debris clog filters/membranes and interfere with enzyme activity. Therefore, such removal of cells is well within the purview of one of ordinary skill in the art when

processing a fermentation broth to obtain a desired protein, i.e. enzyme. Applicant states that Laustens broth cannot be processed according the applicants invention, but it does not appear that applicant actually carried out the claimed method using Lausten's broth. Applicant did not remove cells from the broth when processing according to their claimed invention.

Applicant's arguments pertaining to applicant's distinct objectives, operating conditions, dilution levels and carbon loading are all quite interesting, pertinent and unexpected; however, applicants attention is directed to claim 31. Claim 31 does not possess such distinct and necessary limitations. Applicants conditions which produce these unexpected results are not in claim 31.

Applicants 132 Declaration filed 5/2/2007 fails to show evidence commensurate in scope with the present application. Specifically, applicant has done a comparison, in Fig.1 and 2, of a native enzyme solution, the enzyme solution processed through activated carbon, and that of amylase produced by the method claimed, i.e. dilution and processing through an activated carbon column. It is unclear how one could conclude that such a graph and comparison would provide unexpected results commensurate in scope with applicant's invention. Applicant also does not provide how such activity is being measured, i.e. specific activity measured in the units as known in Enzymology. Applicant should do a side-by-side comparison of the diluted enzyme **without** being processed with activated carbon. There is something to be said of an enzyme solution which has been diluted and it's endogenous inhibitors affecting an increase after dilution, i.e an inhibitor which possesses a low affinity for the enzyme. Changes in

activity or inhibition with dilution are a function of the specific enzyme and amount of enzyme in the initial enzyme solution. Further, activated carbon is a known absorbent, therefore the effect may be explained by the fact that the small molecules present in solution are inhibitors of the enzymes therefore binding to the activated carbon, allowing a more pure enzyme to remain. The examiner's position is that a more effective comparison may include additionally the same diluted enzyme solution not processed with activated carbon compared to the same volume, amount of enzyme diluted solution which has been processed with activated carbon. It is unclear from the Figure legends in applicants declaration which boxes correspond to the white and grey shaded bars.

Applicant arguments in response to the Lausten reference have been considered, however, applicant argues that Lausten discloses an enzyme dilution of 1:1, while this is true, they also disclose at least a two part dilution, see example 2, which teaches at least 2 parts. Applicant also does not show in the submitted Declaration that a dilution of 2:1 is better than or has an unexpected increase of activity compared to a 1:1 dilution. Applicant also argues that Lausten dilutes an enzyme broth which contains cells and in contrast applicants invention contains a broth in which the cells have been filtered out first. Applicant does not specifically claim whether or not the cells are present and/or if they have been filtered out. Thus, applicants arguments are not commensurate in scope with the claimed invention. Applicant argues that the Declaration submitted on 5/2/2007 teaches that filtering an enzyme solution through an activated carbon column does not inherently increase enzyme activity. However, as stated above, the showing in the declaration does not accurately show or compare

“dilution, removal of cells if present, and then contacting with the activated carbon” which results in a surprising enhancement of activity (see p.11 of Arguments). Applicant merely compares the native enzyme solution, the solution with activated carbon and that which has been diluted. Therefore, the arguments are not persuasive.

It is noted that applicant has submitted many documents teaching away from applicants claimed invention, i.e. attempting to overcome the inherency rejection and to show unexpected results.

Regarding applicant's arguments directed towards the Shenoy reference, i.e. that Shenoy does not teaches purification resulting in activity three times higher than the original non-purified glucoamylase, rather compared to a parent strain. While this has been considered, applicant does not specifically claim nor show in the Declaration, purification of an original non-purified enzyme. Applicant actually compares in the declaration dated 5/2/2007, an already purified commercial enzyme to a diluted enzyme purified by the claimed method. Thus, applicant's arguments are not commensurate in scope with the present invention.

Applicant's arguments directed to spectral change continue to be confusing. Applicant is arguing that it is the goal of purification to avoid a structural and spectral change, yet claim a spectral change, which is distinct from the “raw enzyme solution”, as in claim 11,12,13,30. The argument is contradictory. The examiner is well aware of the fact that CD is used after the fact to determine alteration in the structure. Applicant argues that their method is unique in that it leads to a change in protein structure, due to catalytic modification of the protein. Shenoy also teach that the catalytic activity of a

protein, i.e. enzyme is related to its “active” conformation, i.e. secondary and tertiary structure. They state that the ideal purification would preserve protein structure and avoid change in spectral properties, yet claim that their method is unique in that it leads to a change in protein structure, due to catalytic modification of the protein. This argument is not understood.

Applicant argues that the art does not teach or suggest the claimed process of contacting a diluted enzyme solution to activated carbon, in which the cells have been removed. Laustsen by themselves do teach such a process.

In response to applicant previous argument dated 10/19/2006 p.11 of the response, applicant had argued that they do not claim a specific CD spectra, but rather that there had been a change in structure as supported by CD spectra. The examiner’s argument, “Applicant absolutely claims a specific CD spectra in claims 12-15, thus applicants arguments are not supported by the claims.” Applicant now argues in the response filed 5/2/2207 that this statement is not understood and that claims 11-15 show changes in the defined spectra in the claims. Therefore, in claims 12, applicant **does claim** a specific CD spectral range.

Applicant argues that one of skill in the art would not have been motivated to dilute an enzyme prior to purification with activated carbon because the art does not suggest so. However, Laustsen does teach dilution prior to purification. In response to applicant’s arguments that there is no motivation or teaching/suggestion, applicant is advised that KSR forecloses the argument that a specific teaching, suggestion, or motivation is required to support a finding of obviousness. See the recent Board

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decision *Ex parte Smith*,--USPQ2d--,slip op at 20,(Bd. Pat. App & Interf. June 25, 2007)

(citing *KSR*,82 USPQ2d at 1396) (available at

<http://www.uspto.gov/web/offices/dcom/bpai/prec/fd071925.pdf>)

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Conclusion

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to TIFFANY M. GOUGH whose telephone number is (571)272-0697. The examiner can normally be reached on M-F 8-5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jon Weber can be reached on 571-272-0925. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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/Tiffany M Gough/
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